Evaluation of the Effect of Bone Morphogenetic Protein-2 on Stability of Dental Implant

Ali Nihad, B.D.S. (1)
Ali H. A. Al-Hussaini, B.D.S., M.Sc. (2)

ABSTRACT

Background: The healing period for bone–implant contact takes 3–6 months or even longer. Application of Escherichia coli-derived recombinant human bone morphogenetic protein-2 (ErhBMP-2) to implant surfaces has been of great interest on osseointegration due to its osteoinductive potential. The objective of this study was to evaluate the effect of ErhBMP-2 on implant stability.

Materials and methods: A total of 48 dental implants were inserted in 15 patients. Twenty four implants coated with 0.5 mg/ml ErhBMP-2 (study group). The other 24 implants were uncoated (control group). Each patient was received at least two dental implants at the same session. Both groups were followed with repeated implant stability measurements by means of resonance frequency analysis at different time intervals (at the time of surgery, then at 6th and 12th week postoperatively).

Results: there was no obvious statistically significant difference in mean of implant stability quotient ISQ between study and control groups ($P > 0.05$) at time of surgery, whereas the mean of ISQ values at 6th and at 12th week postoperatively were statistically highly significant in the study group compared to the control group ($p < 0.01$).

Conclusion: The results of this study reveal that coating dental implants with ErhBMP-2 increases stability when compared with uncoated implants.

Key words: Bone morphogenetic protein-2, stability, osseointegration, dental implant. (J Bagh Coll Dentistry 2016; 28(3):104-109).

INTRODUCTION

Dental implants and other supportive prostheses are commonly used in dentistry, which rely on a variety of factors to ensure function and survival within the host. The search for a most favorable implant feature is one of the major focuses in the research field to accelerate and improve osseointegration [1].

Multiple design alterations have been attempted, primarily surface modifications, as the implant surface is the first part of the implant that interacts with the bone. Numerous alterations of specific surface features such as structure and chemistry have been successful in speeding up osseointegration at early implantation times which may decrease the total treatment period [1-3].

Recently, further modifications to bioactivate the implant surface using a number of growth factors have also been recommended to induce a deposition of cells with the ability of regenerating the desired tissue [4,5]. An increased proliferation and differentiation of undifferentiated mesenchymal cells, osteoprogenitor cells, and preosteoblasts into osteoblasts may improve bone response and consequently osseointegration of endosseous titanium implants [6]. One particular growth factor; bone morphogenetic protein (BMP), governs the 3 key steps in the osteogenic cascade: chemotaxis, mitosis, and differentiation [7].

(1) Master student. Department of Oral and Maxillofacial Surgery. College of Dentistry, University of Baghdad.
(2) Assistant professor. Department of Oral and Maxillofacial Surgery. College of Dentistry, University of Baghdad.

Bone morphogenetic proteins (BMPs) have shown significant potential to induce bone formation both in ectopic sites [8], and in defect models in different species [9]. A well known osteoinductive factor, the bone morphogenetic protein-2 (BMP-2) appears to possess the highest osteoinductive potential among the BMPs and has been demonstrated to further stimulate local bone formation and osseointegration [10].

In recent years, the regenerative potential of recombinant human BMP-2 (rhBMP-2) has been investigated in various experimental animal studies, including alveolar ridge preservation, sinus floor augmentation, bone augmentation procedures, and periodontal repair [11-14].

Most recently, the effects of rhBMP-2 on the osseointegration of titanium implants have also been investigated in experimental animal studies and revealed an osteoinductive effect [8,15-18].

Huh et al. showed that the ErhBMP-2 coated anodized implants can stimulate bone formation and increase implant stability significantly on completely healed alveolar ridges in dogs [17]. The aim of this study was to evaluate the effect of (ErhBMP-2) coated endosseous dental implants on implant stability.

MATERIALS AND METHODS

Subjects:

Fifteen patients 9 women and 6 men, ranged from 33-65 years old were selected for this study. The patients were referred to the dental implant clinic in the Department of Oral and Maxillofacial

Surgery/ College of Dentistry Teaching Hospital/University of Bagdad. The number of dental implants were 48 fixtures; each patient received at least two dental implants at the same session in the same edentulous region or in a bilaterally symmetric to the median line (split-mouth design), one implant was coated with ErhBMP-2, to serve as a study group, and the other implant was uncoated with ErhBMP-2, to serve as a control group. The study was conducted during the period from October 2014 to November 2015.

Inclusion criteria

Patient's are aged ≥ 18 years with good oral hygiene. Patients have healed edentulous area for at least 6 months after extraction with suitable bone width which not allowed for possibility of dehiscence and/or fenestration. Both study and control groups were provided with the same implant design. The quality of the bone was evaluated by orthopantomograph (OPG).

Exclusion criteria

Heavy smokers (more than 20 cigarettes per day), alcoholism, medically compromised patient, pregnancy, patient with any local pathosis were excluded from this study.

Implant systems:

A total of 48 screw-shaped titanium implants (Implantium® / Dentium® / Seoul / Korea), with a surface modified by TiO_2-large grit, sandblasting and acid etching surface were utilized in the study. The diameter of Ø3.4 mm, Ø3.8 mm, Ø4.3 mm or Ø4.8 mm and a length of 8 mm, 10 mm, 12 mm or 14 mm were placed in the Patient's jaws, who are selected in the study.

Escherichia coli derived recombinant human bone morphogenetic protein-2 (BMP-2):

One vial of rhBMP-2 (0.5 mg /mL; abcam, England) was used in this study. The characteristic features of BMP used in this study are described in table (1).

Ossetell

The implant stability was measured by Ossetell. The SmartPeg® of Ossetell® (Goteborg, Sweden) was screwed to the implant by using SmartPeg Mount then hold the instrument probe close to the top of the SmartPeg® without touching it. An audible sound will be emitted when the instrument senses the SmartPeg® and an ISQ value is generated and shown on the display.

Table 1: Characteristic features of BMP used in this study

Characteristic features	Description
Product name	Human BMP 2 full length protein abcam87065
Nature	Recombinant
Source	E. coli
Form	Liquid
Purity	95% abcam 87065 was purified by using conventional chromatography technique, after refolding of isolated inclusion body in renaturing buffer.
Stability and Storage	Store at -20 to -80 C Preservative ; none
Constituents	10% glycerol, 10 mm sodium citrate, pH 3.5
Manufacture	England abcam

Surgical procedure

Implant surgery stage I (implant placement):

Just right before the surgery, the patient rinsed his mouth with Chlorhexidine mouthwash for 1 minute. Then local anesthesia was given using infiltration technique for the planned area. A flap design on the labial aspect on the implant site was raised. The implant site was prepared by using high torque handpiece at low speed 800 rpm, with sharp drills, intermittent movement and copious external irrigation to prevent excessive thermal injury to the recipient bone.

For study group; 10 µg of ErhBMP-2 carried with micropipette, some of it dropped on surface of titanium fixture and the remaining amount of ErhBMP-2 inserted into the bony hole before insertion of fixture as shown in figures (1, 2 and 3). For control group; implant fixture uncoated with ErhBMP-2.

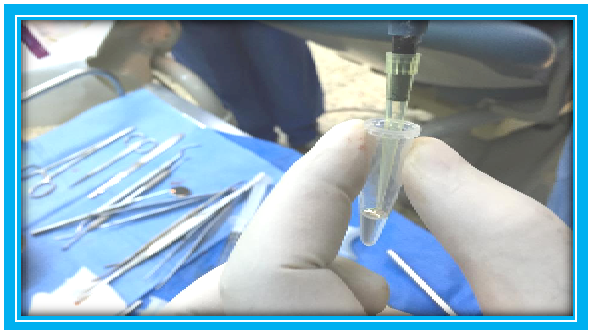

Figure 1: Pipetting of ErhBMP-2

Figure 2: Coating implant fixture with ErhBMP-2.

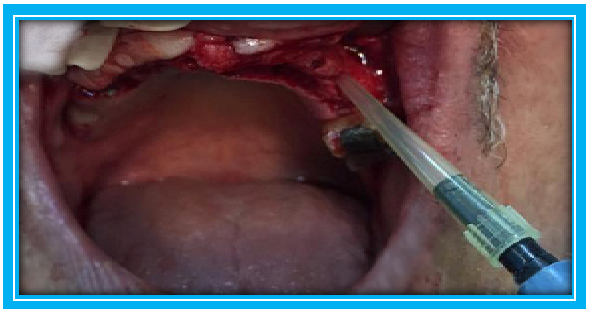

Figure 3: Application of remainingErhBMP-2 to implant preparation sites.

Figure 4: Measurement of primary stability by Ossetell.

After placement of the implant into the bony bed, the implant stability measurement started. Screwing the SmartPeg® of Ossetell® to the implants of study and control groups and two readings of the implant stability quotient (ISQ) values were recorded; in a bucco-lingual and in mesio-distal directions (1st readings) as shown in figure (4).

Implant surgery stage II

After 6 weeks of implant placement, Post-operative OPG was taken to all patients as in figure (5). Under local anesthesia, the uncoverage implant procedure is done with tissue punch drill from Dentium (Dentium, Korea) at 35 rpm. The cover screw was exposed and removed with screw driver and implants secondary stability were measured in the same manner of primary stability measurement for both groups. Depending on the size of implant and the thickness of the overlying gingiva, the proper dimension of the titanium healing abutment (gingival former) was selected and screwed in place. At 12th week after surgery, the gingival former was removed and implants stability was measured in the same manner of primary stability measurement for both groups (2nd readings) (figure 6 and 7). Then the abutments were placed on the implant and take impression and send to prosthodontic laboratory to fabricate final prosthesis as shown in figure (8).

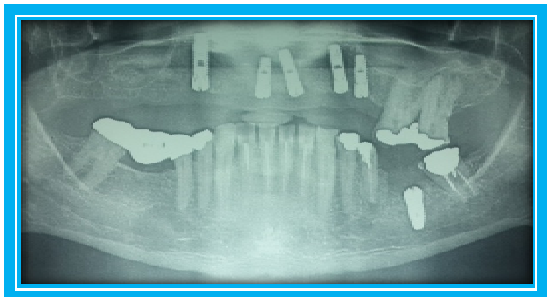

Figure 5: O.P.G was taken 6 weeks after implants placement in the maxillary anterior region.

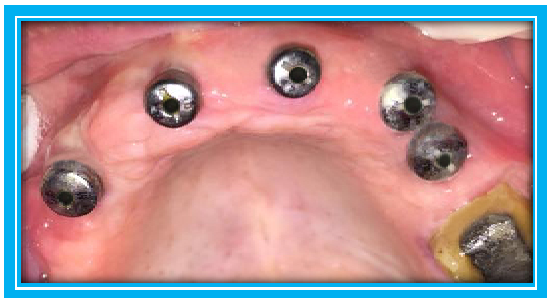

Figure 6: Gingival former after 12 weeks.

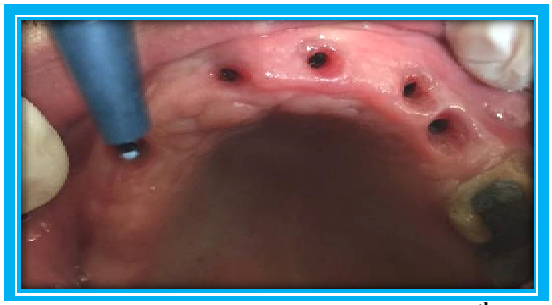

Figure 7: Stability measurement at 12th week of implant placement.

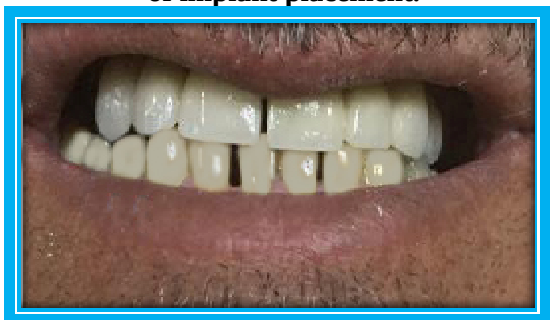

Figure 8: Final prosthesis.

Statistical analyses

Data were translated into a computerized database structure. Statistical analyses were done using SPSS version 21 computer software. Because we had two ISQ measurements for each implant at each time point (mesio-distal and bucco-lingual measurement), average of the two ISQ measurements was used in this results. The independent samples t-test was used to test the statistical significance of difference in mean between the two groups. A significant difference was considered to exist if the p-value was < 0.05.

RESULTS

The effect of healing period on implant stability quotient (ISQ) in control group:

The mean of ISQ reduced by 2.35 units at the 6th week (2nd readings) compared to the primary stability value (1st readings). This mean reduction was statistically non significant. At the 12th week (3rd readings), the mean of ISQ increased by an average of 3.06 units compared to the 6th week, this positive effect was statistically significant.

The change in mean of ISQ after 12 weeks of healing compared to the primary stability value was an average increasing of 0.71 ISQ units, this mean the change was statistically non significant **(table 2).**

Table 2: The mean of ISQ of two perpendicular measurements (bucco-lingual and in mesio-distal directions) after two successive time intervals following surgery in control group

Reading	Descriptive statistics		Reading's difference	
	N	Mean	Mean	p-value
1st	24	66.15	2.35	0.082 (NS)
2nd	24	63.79		
2nd	24	63.79	-3.06	0.011 (S)
3rd	24	66.85		
1st	24	66.15	0.71	0.640 (NS)
3rd	24	66.85		

The effect of healing period on implant stability quotient (ISQ) in study group:

Six weeks after implantation (2nd readings), the mean of ISQ increased by an average of 8.54 units compared to the primary stability readings (1st readings), this positive effect was statistically highly significant (p<0.01).

Twelve weeks postoperatively (3rd readings), the mean of ISQ increased from that of the 6th week by an average of 2.52 units, also this positive effect was statistically highly significant (p<0.01). The total change in mean ISQ after 12 weeks of implantation compared to the primary stability was increase 11.06 units, which is statistically highly significant (P value < 0.01) **(table 3).**

Table 3: The mean of ISQ of two perpendicular measurements (bucco-lingual and in mesio-distal directions) after two successive time intervals following surgery in study group:

Reading	Descriptive statistics		Reading's difference	
	N	Mean	Mean	p-value
1st	24	67.27	-8.54	0.000 (HS)
2nd	24	75.81		
2nd	24	75.81	-2.52	0.001 (HS)
3rd	24	78.33		
1st	24	67.27	-11.06	0.000 (HS)
3rd	24	78.33		

Resonance frequency analysis difference between the study and control groups:

As shown in **table (4)**, There was no obvious or statistically significant difference in mean of ISQ between the two groups at time of surgery (P = 0.549), the mean values of ISQ at time of surgery was 66.15 for the control group and 67.27 for the study group.

At 6th week postoperatively, the difference in mean ISQ was greater in the study group by 12.02 units compared to the control group, this intervention effect was highly significant and evaluated as a strong effect (p<0.01). The difference in mean of ISQ units after 12 weeks of surgery was also highly significant between the study group when compared to the control group and the treatment effect was evaluated as a strong effect (p<0.01,) (figure 9).

Table 4: Descriptive statistics of ISQ difference between the study and control groups

Reading	Groups	Descriptive statistics		Group's difference	
		N	Mean	Mean	p-value
1st	Control	24	66.15	-1.13	0.549 (NS)
	Study	24	67.27		
2nd.	Control	24	63.79	-12.02	0.000 (HS)
	Study	24	75.81		
3rd	Control	24	66.85	-11.48	0.000 (HS)
	Study	24	78.33		

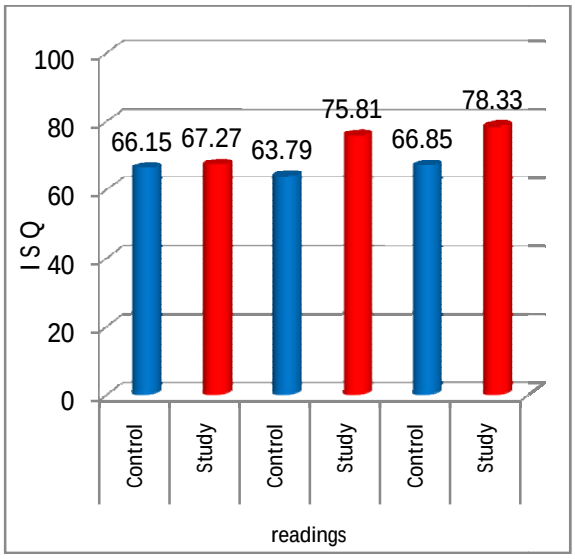

Figure 9: Resonance frequency analysis values of the study and control groups.

DISCUSSION

The ISQ values for the study group were consistently greater than the control group implants at all time points. The values were statistically non significant at time of implant placement, but the values were statistically highly significant after 6 and 12 weeks following implant placement. This may be due to the use of ErhBMP-2 on surface of dental implant which may decrease the period for bone–implant osseointegration and increase secondary stability (biological fixation). The results were in agreement with Huh et al who found that a small difference in ISQ value was observed in the control group from the time of surgery to 8 weeks after surgery, whereas the experimental groups (implants dip–coated by ErhBMP-2 0.75 mg/ml, 1.5 mg/ml) showed a significant increase in ISQ value compared with control group [19].

The results were also in agreement with Huh et al who found that the ISQ values were significantly higher in the implants immersed in a protein solution (BMP group) than in the control group at 8 weeks after implant placement on completely healed alveolar ridges in dogs ($p < 0.05$) [17].

Similar outcomes were reported in humans after implantation of BMP in extraction sockets with titanium microscrews. Despite the small number of samples in the group, the BMP was related to larger amounts of viable lamellar bone and woven bone compared with the other treatments [20].

The results disagree with Salata et al who found that there were no statistically significant differences between resonance frequency analysis (RFA) measurements of rhBMP-2 group and other groups [21].

The method, concentration of (0.5 mg/ml) and dosage of (10 µg) utilized in the present study was effective in increasing implant stability. In regard to method of application and form of ErhBMP-2 which were used in this study, the implant surfaces were coated with liquid form of ErhBMP-2 by using micropipette. This method may agree with Yoo et al, who showed that the application of the osteogenic rhBMP-2 to titanium implant surfaces by immersion in protein solution before implant installation significantly increased bone-implant contact (BIC) and bone area fraction occupancy as a result enhanced osseointegration [18]. The method of application and form of rhBMP-2 which were used in this study may also agree with studies of other researchers [8,17,19,22-24] **which showed that** rhBMP-2 dip-coated onto titanium implant surfaces induced clinically relevant local bone formation and osseointegration.

Concerning the concentration used in this study, Tatakis et al reported that there were no significant differences in bone formation and osseointegration in beagle dogs with rhBMP-2 at concentrations of 0.05, 0.1, and 0.2 mg/ml [25]. In contrast, Becker et al concluded that rhBMP-2 immobilized by covalent and non covalent methods on chromosulfuric acid -treated titanium implant surfaces seemed to be stable and promoted direct bone apposition in a concentration dependant manner [26].

The dose used in this study was 10 µg similar to the dose used by Hall et al, who used implants adsorbed with 5, 10, or 20 µg rhBMP-2 were implanted subcutaneously into the ventral thoracic region in 5-week-old male Long Evans rats. At day 14 post-surgery, the histologic analysis showed greater amounts of bone formation, osteoblastic cells, osteoid, marrow, tissue encapsulation, vascularity, and bone voids for implants adsorbed with 10 and 20µg rhBMP-2 [27].

The dose used in this study also similar to the dose used by Bessho et al who observed that the reverse torque strength was statistically highly significant for BMP stimulated implants in 10 µg than for non-BMP stimulated implants after 3 weeks and 12 weeks of implantation respectively. Histomorphometric evaluations indicated more bone contact with the BMP stimulated implants compared to the controls after 3 weeks implantation [28].

In conclusion, the results of the present study suggest that ErhBMP-2 coated sandblasted acid-etched endosseous dental implants can increase implant stability significantly on completely healed alveolar ridges in human.

REFERENCES

1. Coelho P, Granato R, Marin C, Teixeira H, Suzuki M, Valverde G, Janal M, Lilin T, Bonfante E. The effect of different implant macrogeometries and surface treatment in early biomechanical fixation: An experimental study in dogs. J Mech Behav Biomed Mater 201; 4:1974–81.
2. Le Guéhennec L, Soueidan A, Layrolle P & Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23(7): 844-54.
3. Novaes Jr A, Souza S, Barros R, Pereira K, Iezzi G & Piattelli A. Influence of implant surfaces on osseointegration. Braz Dent J 2010; 21(6): 471-481.
4. Urist M, DeLange R, Finerman G. Bone cell differentiation and growth factors. Science 1983; 220: 680–6.
5. Wozney J, Rosen V, Byrne M, Celeste AJ, Moutsatsos I, Wang E. Growth factors influencing bone development. J Cell Sci Suppl 1990; 13:149–56.
6. Chappard D, Aguado E, Huré G, Grizon F, Basle M. The early remodeling phases around titanium implants: a histomorphometric assessment of bone quality in a 3-and 6-month study in sheep. Int J Oral Maxillofac Implants 1999; 14(2): 189-96.
7. Reddi A. Bone and cartilage differentiation. Curr Opin Genet Dev 1994; 4(5): 737-44.
8. Cole B, Bostrom M, Pritchard T, Sumner D, Tomin E, Lane J, Weiland A. Use of bone morphogenetic protein 2 on ectopic porous coated implants in the rat. Clin Orthop Relat Res 1997; 345: 219-28.
9. Moghadam H, Urist M, Sandor G, Clokie C. Successful mandibular reconstruction using a BMP bioimplant. J Craniofac Surg 2001; 12: 119–27; discussion 128.
10. Cheng H, Jiang W, Phillips F, Haydon R, Peng Y, Zhou L, He T. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg 2003; 85(8): 1544-52.
11. Boyne P, Lilly L, Marx R, Moy P, Nevins M, Spagnoli D, Triplett R. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. J Oral Maxillofac Surg 2005; 63(12): 1693-707.
12. Fiorellini J, Howell T, Cochran D, Malmquist J, Lilly L, Spagnoli D, Nevins M. Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. J Periodontol 2005; 76(4): 605-13.
13. Sigurdsson T, Nygaard L, Tatakis D, Fu E, Turek T, Jin L, Wikesjö U. Periodontal repair in dogs: evaluation of rhBMP-2 carriers. Int J Periodontics Restorative Dent 1996; 16(6): 524-37.
14. Sailer H, Kolb E. Application of purified bone morphogenetic protein (BMP) preparations in cranio-maxillo-facial surgery: reconstruction in craniofacial malformations and post-traumatic or operative defects of the skull with lyophilized cartilage and BMP. J Craniomaxillofac Surg 1994; 22(4): 191-9.
15. Kawai T, Mieki A, Ohno Y, Umemura M, Kataoka H, Kurita S, Urist MR. Osteoinductive activity of composites of bone morphogenetic protein and pure titanium. Clin Orthop Relat Res1993; 290: 296-305.
16. Vehof J, Mahmood J, Takita H, Hof M, Kuboki Y, Spauwen P, Jansen J. Ectopic bone formation in titanium mesh loaded with bone morphogenetic protein and coated with calcium phosphate. Plast Reconstr Surg 2001; 108(2): 434-43.
17. Huh J, Kim S, Kim H, Kang S, Choi K, Jeong C, Shin S. Effects of anodized implants coated with Escherichia coli-derived rhBMP-2 in beagle dogs. Int J Oral Maxillofac Surg 2012; 41(12): 1577-84.
18. Yoo D, Tovar N, Jimbo R, Marin C, Anchieta R, Machado L, Coelho P. Increased osseointegration effect of bone morphogenetic protein 2 on dental implants: An in vivo study. J Biomed Mater Res Part A 2014; 102(6): 1921-7.
19. Huh J, Park C, Kim S, Shim K, Choi K, Kim S, Shin S. Alveolar ridge augmentation using anodized implants coated with Escherichia coli–derived recombinant human bone morphogenetic protein 2. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(1): 42-9.
20. Becker W, Clokie C, Sennerby L, Urist M, Becker B. Histologic findings after evaluation of different grafting materials and titanium micro screws into extraction sockets: case reports. J Periodontol 1998; 69: 414–21.
21. Salata L, Burgos P, Rasmusson L, Novaes A, Papalexiou V, Dahlin C, Sennerby. Osseointegration of oxidized and turned implants in circumferential bone defects with and without adjunctive therapies: an experimental study on BMP-2 and autogenous bone graft in the dog mandible. Int J Oral Maxillofac Surg 2007; 36: 62–71.
22. Lan J, Wang Z, Shi B, Xia H & Cheng X. The influence of recombinant human BMP-2 on bone–implant osseointegration: biomechanical testing and histomorphometric analysis. Int J Oral Maxillofac Surg 2007; 36(4): 345-9.
23. Wikesjö U, Qahash M, Polimeni G, Susin C, Shanaman R, Rohrer M, Hall J. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations. J Clin Periodontol 2008; 35(11): 1001-10.
24. Leknes K, Yang J, Qahash M, Polimeni G, Susin C, Wikesjö U. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: radiographic observations. Clin Oral Implants Res 2008; 19(10): 1027-33.
25. Tatakis D, Koh A, Jin L, Wozney J, Rohrer M, Wikesjo U. Peri-implant bone regeneration using recombinant human bone morphogenetic protein-2 in a canine model: a dose-response study. J Periodontal Res 2002; 37: 93–100.
26. Becker J, Kirsch A, Schwarz F, Chatzinikolaidou M, Rothamel D, Lekovic V, Jennissen, H. P. Bone apposition to titanium implants biocoated with recombinant human bone morphogenetic protein-2 (rhBMP-2). A pilot study in dogs. Clin Oral Investig 2006; 10(3): 217-24.
27. Hall J, Sorensen R, Wozney J, Wikesjö U. Bone formation at rhBMP-2-coated titanium implants in the rat ectopic model. J Clin Periodontol 2007; 34(5): 444-51.
28. Bessho K, Carnes D, Cavin R, Chen H, Ong J. BMP stimulation of bone response adjacent to titanium implants in vivo. Clin Oral Implants Res 1999; 10: 212–18.